THE FATE OF RADIOPHOSPHORUS INGESTED BY HABROBRACON FEMALES [1, 2]

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In a previous publication the authors introduced the subject of the effects of phosphorus-32 on the development and heredity of offspring from adult insects given one feeding of the radioactive material (Grosch and Sullivan, 1952). The organism considered was the "ectoparasitic" wasp known in genetic literature as *Habrobracon juglandis* (Ashmead). Attention was directed to the curves representing daily egg production and to the hatchability of the eggs laid by samples of wasps after feedings at various levels of radioactivity. Subsequently we have attempted to clarify dosage relationships. In particular our desire has been to arrive at a basis for determining the dosage of radiation to which an egg may be subjected, and the present publication is concerned with some of the complications which need to be considered.

Two sources of beta radiation contribute to the dosage to which the egg contents are exposed: (1) the P^{32} in the soma of the female, and (2) the P^{32} herein shown to become incorporated into the egg. Therefore, in reference to (1), the present investigation of the consequences of ingestion of radiophosphorus was concerned with (a) observing change in the detectable radiation from wasps per unit time, an index of which is the biological half-life, and (b) illustrating changes in the distribution of radiophosphorus over the significant part of the ovulation period. Demonstrated in reference to (2) are (a) the channels whereby P^{32} may be lost by a female wasp and (b) the period during which a relatively high egg radioactivity obtains. Accordingly we are presenting the first study on insects which gives detailed chronological attention to excretory and ovipositional loss of radioactive substance subsequent to a single feeding of such material.

In addition to contributing toward a general understanding of what happens to ingested radioactive phosphorus, the present paper provides an explicit explanation of the upward slope of hatchability curves in the first week shown in the preceding report (Grosch and Sullivan, 1952). In reference to the 1952 report it should be mentioned that millicuries were erroneously indicated in the legends of several figures. Microcuries were intended throughout.

Materials and Methods

Virgin females from Habrobracon Stock No. 33 were fed mixtures of honey and P^{32} at various levels of radioactivity lower than the sterilization dose of 200 μc

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per gram of mixture. Each female was given one feeding only. Biological aspects of ingestion experiments and feeding technique have been treated in detail previously (Grosch and Sullivan, 1952).

After feeding, information on the distribution and excretion of radioactive phosphorus at desired intervals was obtained from the counting equipment of the Department of Experimental Radiology, Marine Biological Laboratory, Woods Hole, Massachusetts. In particular we wish to thank Mones Berman for helpful advice and direction. Counts were determined thrice in reproducible position for each specimen. Thus the starting point of analysis was a calculation of the average of three determinations.

Nearly 300 wasps were employed in obtaining daily measurements of radioactivity on the following: (1) whole animals, anaesthetized or dead, (2) separated parts, anterior and abdomen after transection at the petiole, (3) ovaries, a few dissections only, (4) eggs, in groups and as individuals, and (5) excreta. In following daily change in the radioactivity of the organism or in checking the radioactivity of a part or product, the wasps were handled in groups of various sizes as will be detailed below when presenting results.

The technique devised for investigating the radioactivity of excreta is new. It involves maintaining the female in a punctured No. 3 gelatin capsule with one host caterpillar (*Ephestia*). The puncture in the capsule wall seemed necessary to avoid oxygen depletion. Periodic transfer of the female is made at desired time intervals, one day in the present study, whereupon female and caterpillar with deposited eggs are removed from the capsule. The caterpillar is examined microscopically for purposes of transferring any excreta (by dissecting needle) from caterpillar to the capsule thus adding to the excreta deposited on the walls. The capsule is then digested in dilute HCl in a steel planchette. After dissolution the sample is evaporated and counted.

Radioautographs made in our laboratories by Mrs. Margaret T. MacLeod have proved helpful in interpreting distribution results and reference is made to them below. Standard procedures recommended by the Eastman Kodak Company were employed in making the autographs on their Nuclear Track Plates (beta) of the 1×3 in. size with $10\,\mu$ emulsion thickness.

Results

Radioactivity of the organism

The time taken for the radioactivity of a wasp to fall to half of its initial value as the result of elimination and radioactive decay has been determined to be between four and five days as shown in Figures 1 and 2. This convenient index is known as the biological half-life. In addition, other details pertaining to change in radioactivity of a wasp can be discussed in reference to the figures.

Figure 1 presents comparatively the loss in radioactivity for three categories of whole animals: (1) killed immediately, (2) living but not ovipositing, and (3) living and ovipositing. Wasps of category (2) are infrequently seen and their ascribed lines are based only upon single animals. Also as shown by the ending of the pertinent lines, the life-span of non-laying wasps is short. The curves for non-layers are very useful in demonstrating the relatively slow loss of radioactivity when eggs are not being deposited. In categories (1) and (3) results for six

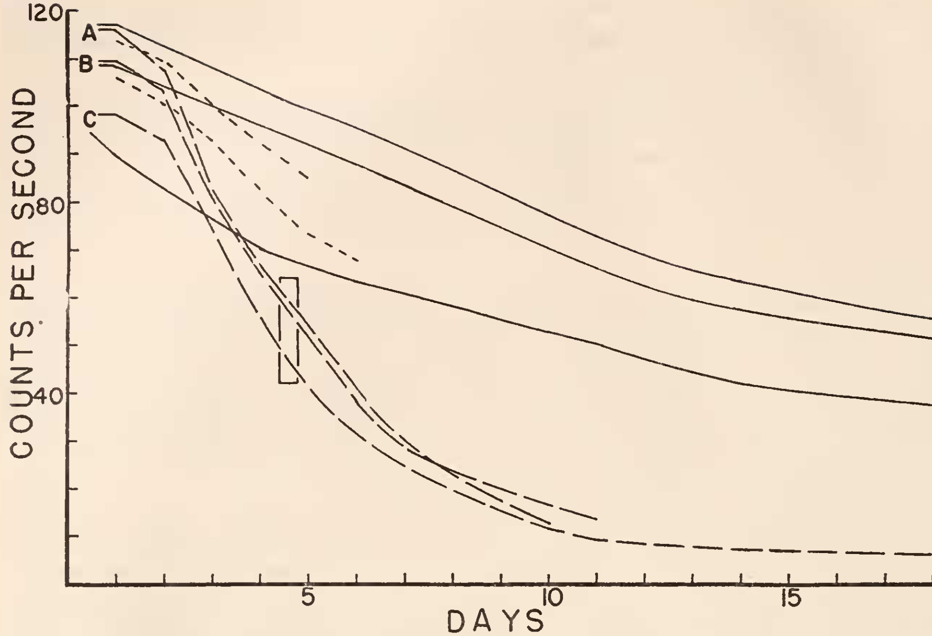

FIGURE 1. The radioactivity in mean counts per second when adult female Habrobracon are followed subsequent to ingestion of P^{32}. The solid lines are for wasps killed immediately after the single feeding. The short dashes are for live wasps not depositing eggs. The long dashes are for live wasps which are depositing eggs. The designation of the curves as A, B, and C on the basis of initial count is explained in the text. The rectangle drawn between the fourth and fifth day marks the time at which biological half life has been attained.

killed and nine ovipositing wasps, respectively, were obtained. The data from individuals in each category were grouped separately according to the level of radioactivity found in their initial counts:

Group	Initial count (c/sec.)	Individuals per group (1) Killed	(3) Ovipositing
A	110–119	2	3
B	100–109	2	3
C	90– 99	2	3
		6	9

The six curves obtained by plotting the group means on successive days are shown in Figure 1. Since all females were allowed to feed once from the same honey-P^{32} mixture, the different levels of radioactivity found in initial counts are ascribed to chance variation.

To obtain merely a decay-plus-loss curve for ovipositing animals, all nine ovipositing animals could have been lumped and the mean value compared with that from a grouping of the six dead wasps. However such a procedure obscures the detail of the crossing of lines A and B shown between days 7 and 8. An explanation of this detail seems to lie in the number of eggs deposited. Whereas the wasps represented by line A had deposited an average cumulative total of 63 eggs per animal by the sixth day, those of line B had deposited only 61.6 eggs per animal. Line C which runs almost parallel to B represents females of quite similar egg productivity (59.9 eggs, cumulative total per animal). The implication of course is that the more eggs an animal lays per unit time, the more rapid may be its loss of radioactivity. The difference in slope between non-laying and laying live wasps points to the egg as a highly significant channel of P^{32} loss. Direct evidence of egg radioactivity is treated in the following section.

Figure 2 presents results based on larger numbers of animals in verification of the biological half-life. Thirty live ovipositing animals were followed for seven days. These animals comprised three groups which reflect the feeding of three

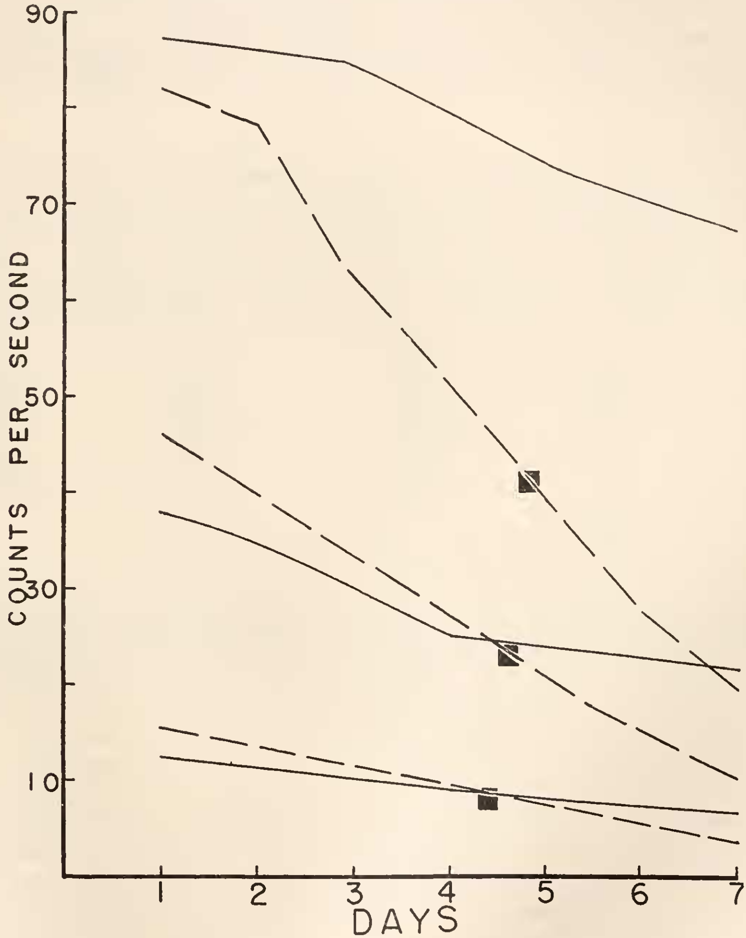

FIGURE 2. Verification of the results of Figure 1 using larger samples of wasps at three levels of radioactivity. The solid lines are for wasps killed immediately after feeding. They demonstrate measurable decay of P^{32} with a half-life extrapolating to between 14 and 15 days. The broken lines refer to egg-laying wasps with squares drawn to mark biological half life reached between four and five days.

different radioactive honey mixtures. Ten wasps were obtained therefrom with initial counts of less than 30 per second, 10 between 30 and 60 counts per second, and 10 between 60 and 100 counts per second. The group means of these live ovipositing wasps have been plotted as three broken lines to be compared with the solid lines which represent the mean values for 7 killed wasps numbering 2, 2 and 3 dead individuals per respective range of initial count. Here too it is demonstrated that the total radioactivity of an animal is related to the number of eggs deposited. By the fourth day the wasps counting less than 30 c/second had deposited an average total of 33.4 eggs per animal, those between 30 and 60 c/second had deposited an average total of 31.5 eggs per animal and the 60 to 100 c/second group had deposited an average total of only 24.2 eggs per animal. As indicated in Figure 2 the animals depositing the greatest number of eggs (wasps grouped by initial count of less than 30 per second) reached biological half-life first, while those depositing the fewest eggs (initial count more than 60 per second) reached biological half-life last.

Radioactivity of eggs

Figure 3 presents the results of following radioactivity for a week or more by taking GM counts of the eggs produced each day during that length of time. Depending upon the stage of her production cycle, a female deposits from 5 to 20 eggs per day. This number of eggs, whatever it totalled, was spread out on a planchette and counts per second were determined. For purposes of summary, the data which reflect feeding with four different honey-P^{32} mixtures, were grouped as follows:

Group	Number wasps per group	Initial count of wasps c/sec.	Total eggs per group	Max. level c/sec. per egg
A	9	above 90	393	10.99
B	19	60–90	1403	7.28
C	17	30–59	1133	5.32
D	5	5–15	819	2.09
Totals	50		3748	

The mean counts per egg for each group on successive days are plotted in Figure 3. A very definite relationship is thus shown between the radioactivity of females and of the eggs they deposit. Curve A reaches and continues at the highest values. Curves B, C, and D reach and continue at lower values in the order of the radioactivity of the wasps which deposited the eggs.

The curves for egg radioactivity are shown to have very typical shape. The level of activity rises sharply to a peak during the first two days, the maximum level obtained in each case. It then drops toward a relative low reached on the fifth or sixth day. In an additional experiment, not graphed, daily counts on eggs from a sample of 21 wasps were begun on the fourth day and made subsequently for a week, which proved tenable the supposition that the level of radioactivity of eggs continues to fall slowly. A total of 1075 eggs was considered by daily deposited groups in this experiment.

In further experiments, counts were made on single eggs of all those deposited by individual wasps on particular days subsequent to the ingestion of radioactive material. This was an attempt to obtain information on the variability in radioactivity of eggs within given days. Records of the radioactivity of 212 eggs were ob-

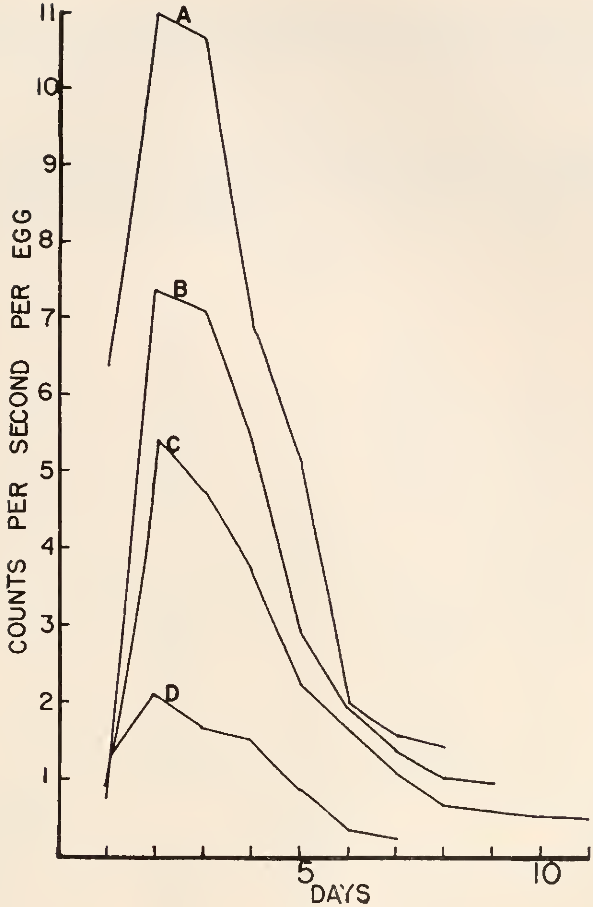

FIGURE 3. The radioactivity of Habrobracon eggs laid by samples of wasps after feeding once on honey containing P^{32} at zero days. Each line represents calculations of the arithmetic average for single eggs derived from GM counts per second on accumulations of eggs representing the daily production from individual wasps. The different level reached on day two reflects the initial level of radioactivity of the wasps depositing the eggs, and the designation A, B, C, and D is in order from high to low.

tained, representing conditions over the first five days of egg production. A variety of dosages were considered which resulted in wide differences in the means. The mean itself was not of primary interest here but rather the degree of variation around the mean to be represented as a comparative or relative measure. Such a measure is furnished by the coefficient of variation. Summarization is given in Table I. It will be noted that the comparisons are made between samples of approximately the same size, hence it is unlikely that S.D.'s and C.V.'s are varying because of chance inclusion of extreme variates in unequal sized samples. On the first day there is appreciable variation in radioactivity as shown by standard errors which are relatively large when compared with the mean. This is expressed in consistently higher coefficients of variation than are seen in subsequent days. Data for second and third days show variability similar to each other. Because of the time-consuming aspects of this type of investigation the eggs from few wasps could be handled on the fourth and

TABLE I

Summary of radioactivity data for single eggs in counts per second

Number of eggs	Mean	Standard deviation	Coefficient of variation
Day 1			
5	3.97	1.57	39.5
7	8.41	3.06	36.3
7	2.68	0.92	34.3
5	6.49	2.17	33.4*
7	8.45	2.36	27.9
7	2.78	0.58	20.8
5	37.96	5.80	15.3
5	24.46	2.47	10.1
Day 2			
9	14.18	3.09	21.8*
6	10.13	1.33	13.1
6	10.13	1.16	11.5
8	11.42	1.07	9.3
8	11.41	1.03	9.0
11	12.57	1.04	8.2
12	12.46	1.01	8.1
5	14.40	0.75	5.2
Day 3			
9	11.39	2.99	26.2*
11	10.79	2.62	24.2
8	45.61	5.96	13.0
14	20.10	2.36	11.7
8	13.18	1.22	9.2
4	36.85	3.02	8.2
8	30.62	1.89	6.1
Day 4			
6	9.27	2.15	23.1
4	46.05	5.19	11.2
5	5.96	0.62	10.4*
3	10.91	0.66	6.0
8	6.33	0.26	4.1
Day 5			
6	5.69	0.62	10.9
6	6.53	0.27	4.1*

fifth day; too few to permit conclusions to be drawn. After the fifth day, individual eggs count so very slowly that investigation of the radioactivity of single eggs had to be discontinued.

Admittedly it would have been ideal to have all records provided by the same animals followed for the whole period. However, in the absence of an automatic feeder device for the equipment used to measure radioactivity, it was humanly impossible to carry out the ideal design of experiment. Only in one case were the eggs from the same animal examined over the whole five day period. These are indicated on the Table by asterisks. Here there is suggested progressively less variation in successive days.

Radioactivity of excreta

The radioactivity of the excreta collected by the gelatin capsule method was measured daily for each wasp of a sample of twenty-five fed from the same honey-P^{32} mixture. Before and after the four-day duration of the experiment the radioactivity of each wasp was also measured. By correcting for isotope decay, the initial radioactivity of each wasp and of its excreta were put on a common basis with the final radioactivity of each wasp. The amount of loss was then calculated (by subtraction) for comparison with the total amount (addition) of radioactivity of the excreta. Table II presents a summary of these data for actively ovipositing wasps and gives the comparison as a percentage. Since it was the comparison which was particularly desired the data are organized with reference to percentage by a listing in

TABLE II

A comparison of the radioactivity of excreta with the total radioactivity lost in four days by ovipositing Habrobracon

Total radioactivity of excreta in c/sec. corrected for decay	Loss in radioactivity not due to decay c/sec.	Per cent of loss through excreta
10.18	48.22	21.1
5.81	25.11	23.1
10.78	44.63	24.2
9.47	36.95	25.6
15.67	55.76	27.6
19.69	62.75	31.4
9.59	29.51	32.5
5.94	17.30	34.4
11.99	30.55	39.2
10.04	25.47	39.4
7.99	19.93	40.1
10.47	25.98	40.3
15.41	37.81	40.7
23.25	55.96	41.5
22.00	52.70	41.7
8.03	17.61	45.5
9.42	19.69	47.8
5.67	11.51	49.2
7.36	14.57	50.5
13.02	24.20	53.7
19.93	36.82	54.2
25.27	43.49	58.1
8.38	13.75	60.9

order from the lowest to the highest. A consideration of the distribution of percentages in Table II demonstrates that the mean, median and mode fall near 40%, a value which may be taken provisionally to represent the average loss of radioactive phosphorus to be expected to occur by way of the excreta for ovipositing females. Data for two females which laid few or no eggs have been omitted from the Table. Excreta accounted for 82% and 100% of their radioactive loss.

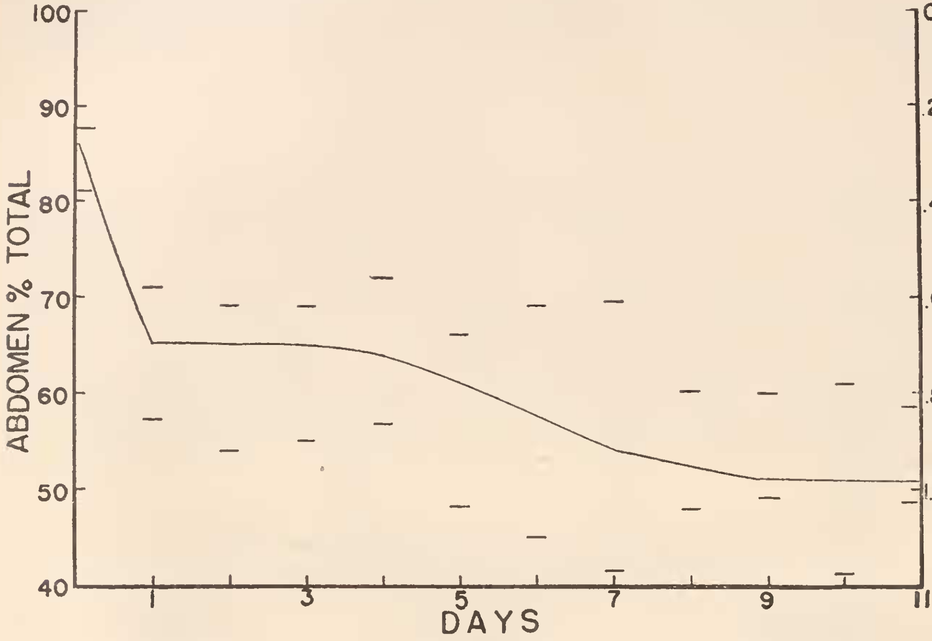

FIGURE 4. The distribution of P^{32} in Habrobracon females subsequent to a single feeding of the honey mixture. The mean value is plotted for the per cent of the total radioactivity demonstrable for the abdomen and the daily range is deliminated by dashes. Included on the right is a scale for interpreting the same results in terms of an anterior/posterior ratio.

Distribution of P^{32}

Transection experiments summarized in Figure 4 demonstrate that shortly after feeding, the abdomen contains a major amount (86%) of the radioactive material. This of course is to be expected since the crop, an organ which functions in immediate storage of food, has an abdominal location. Within twenty-four hours the proportionate distribution has changed so that only about 65% of the active material is abdominal. As shown, this situation remains constant during four successive days corresponding to the period during which the biological half-life is reached and during which appreciable amounts of radioactive material are egested and incorporated into deposited eggs. Subsequently, the abdominal radioactivity falls off so that from the seventh day forward there is only slightly more than 50% of the total radioactivity demonstrable as abdominal in a majority of animals. These conclusions are based upon transections of 134 females, of which 5 to 15 were sacrificed per day. Eggs laid each day were recorded and cumulative egg production for each female was calculated therefrom. Although oviposition has been shown above to be a very significant channel for exit of radioactive material and therefore a very important factor in the regulation of the total radioactivity of the animal, egg laying does not appear to be significant in determining P^{32} distribution.

Attempts to show a correlation between total eggs produced and proportionate distribution of P^{32} have failed. The evidence as given by scatter diagrams will not be presented. Instead the following selected examples will serve to demonstrate the point that although there may be appreciable differences in the number of eggs laid by females, however, provided an equivalent amount of time has elapsed since feeding, a very similar distribution of radioactive material may be expected:

Day	Abdominal per cent of radioactivity	Cumulative total eggs
7	53.67	94
	53.62	115
9	52.73	98
	52.82	56
11	52.23	183
	52.40	71

Apparently egg production is much less important in bringing about distribution than uninvestigated non-specified metabolic activities which appear to require elapsed time. Consistent with these conclusions is the fact that ovaries dissected out of adult females show very slow counts after biological half-life has been passed. In the present investigation, dissected ovaries were found to contain less than 33% of the abdominal radioactivity and one egg-laying female on the eighth day carried only 7% of her abdominal radioactivity in the ovaries.

The conclusion that physiological activities other than egg production are importantly involved in the distribution of radioactive phosphorus raises the question of where the material is present. The gonads apparently do not function as a storage depot but rather as disposal units which draw upon the reservoirs in the organism for the P^{32} to be incorporated into eggs. Thus distribution of radiophosphorus in Habrobracon is viewed as mobile and adjustable, an approach entirely acceptable on the basis of turnover studies in other organisms. One of the most definite of the general results emerging from tracer research is the demonstration of the dynamic character of molecules involved in metabolism (Kamen, 1947).

Radioautographs of Habrobracon females fed P^{32} are in accordance with these views in showing the trophocytes as the most radioactive elements of the gonads, while in addition other wasp tissues become particularly radioactive such as the muscles, the midgut epithelium, the brain and the central nervous system. Observations of radiophosphorus content of the tissues enumerated are consistent with reports on radioautographs of other insects (Lindsey and Craig, 1942; Irwin, Spinks and Arnason, 1950). Incidentally, because we are using P^{32} in amounts larger than tracer doses, the density of silver granules is great and resolution is lacking for some time after feeding. In fact, distinguishable details on wasp radioautographs were not clear unless the animal was sacrificed several days after feeding and our best radioautographs were made after biological half-life. The only detail discernibly distinct in radioautographs made one day after P^{32} feeding is the lack of radioactivity for the contents of the digestive tract in the region of the crop and stomach.

Discussion

A consistent picture emerges here of the loss of most of an ingested quantity of P^{32} within a few days after a single feeding of the radioactive material. Furthermore three lines of evidence point to the egg as a major channel whereby the isotope is eliminated from the fed female. In the first place, a comparison of curves in Figure 1 indicates the rapidity with which ovipositing wasps lose radioactivity in comparison to non-laying wasps. Secondly, the measurements of excretal radioactivity demonstrate that excretory deposits account for only around 40% of the loss in radioactivity. Finally, there is direct evidence from the demonstrated radioactivity of the eggs themselves. Thus an individual egg from a wasp fed P^{32} is subjected not only to the beta rays from the radioactive tissues and body fluids before laying, but irradiation is continued after deposit from the P^{32} incorporated into the egg.

Eggs laid the first day may be expected to have been in Metaphase I, a susceptible stage (Whiting, 1945), and hence influenced by maximum exposure to emanations from the soma of the mother. However, those laid subsequently up to about the sixth day were progressing through Prophase I, a relatively resistant stage, when the mother is most radioactive (Day 1). A great difference has been demonstrated between the eggs of day 2 and those of day 5 in the amount of egg-incorporated radiophosphorus. Curve A of Figure 3 shows that eggs on the second day after feeding can have as much as seven times the incorporated amount of radioactive material demonstrable a few days later. The period when incorporated P^{32} is at a high level is also when hatchability was found to be low particularly after a feeding of highly radioactive mixture (Grosch and Sullivan, 1952). Subsequently in the period when radioactivity falls off, the hatchability increases appreciably. We therefore believe now that egg radioactivity is most important in explaining hatchability during the 1- to 5-day period.

Eggs laid after the fifth day are not appreciably radioactive. Either initial exposure as mitotically active oogonia or total accrued dosage may be more significant in influencing their hatchability than the incorporated amount of radioisotope. In any event, it has become evident that eggs laid at different times by the same radiophosphorus-fed animal become exposed to quite different dosages of beta radiation from various sources.

The present authors are especially impressed with the concept of a gamete which carries built-in radiation equipment of particular potency in eggs laid early in the first week. Any susceptible stage in oogenesis or embryological development subsequent to incorporation may be subjected to ionizing radiations from a most intimate source, perhaps even from the chromosomes themselves.

There may be important genetic implications of the present results in an extension to what may occur in economically important vertebrates. For one thing, large amounts of radioactive phosphorus have been demonstrated to be removed from the physiological interior of the domestic fowl by way of the egg (Lorenz *et al.*, 1942). However attention has apparently not been directed to either the hatchability or the mutational potentialities of such radioactive products. Instead, interest has been devoted to applying tracer techniques to the physiological aspects of the treated adult rather than to investigating developmental or genetical consequences in the offspring (Entenman *et al.,* 1938; Hevesy, 1948; Lorenz *et al.*, 1942).

On the other hand, since there is no equivalent shell featuring inorganic constituents, it does not seem profitable to anticipate Habrobracon physiology on the basis of poultry work except in broad general terms. If the extension from vertebrate to invertebrate is valid, the element would soon make its appearance as organic phosphorus and as such be incorporated into eggs. The two days between feeding and the peak radioactivity of eggs seem to be an adequate period elapsed for synthesis.

Summary

1. The biological half-life of radiophosphorus in egg-laying Habrobracon females is four to five days.

2. A majority of the P^{32} lost, about 60%, is by way of deposited eggs. This is based upon excretory studies using a newly devised technique, and upon comparisons of laying and non-laying wasps. Direct evidence of the radioactivity of the eggs is also presented.

3. The radioactivity of eggs rises to a peak reached the second day after feeding. Subsequent to the third day, it drops sharply to a relatively low plateau.

4. The variability in radioactivity from egg to egg is greatest in the first day. It may prove to be less each succeeding day.

5. During the first day almost 90% of the ingested radioactive material is abdominal due to the location of the crop. From the second to the fourth day about 65% of the radioactivity in the female is abdominal. Subsequently abdominal radioactivity approaches 50% of the total. Of these proportions less than one-third may be gonadal.

6. Although it is an important factor in the regulation of total radioactivity, egg laying has not been found significant in determining P^{32} distribution. A mobile, adjustable situation is indicated.

7. The shape of the hatchability curve previously reported for eggs from females fed P^{32} is interpreted for the first five days on the basis of gamete-incorporated radiophosphorus. Subsequently, initial exposure and accrued total dose may have greater significance.

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